

**Amendments to the Claims**

1-10. (Canceled)

11. (New): A method for identifying a selective blocker of a persistent Na<sup>+</sup> channel whereby the method comprises the steps of:

- a) providing a test sample 1 comprising
  - i) a Na<sup>+</sup>-free physiological buffer;
  - ii) a voltage-sensitive fluorescence dye;
  - iii) a cell having a K<sup>+</sup> channel, a transient Na<sup>+</sup> channel and a persistent Na<sup>+</sup> channel;  
and
  - iv) a potential Na<sup>+</sup> channel blocker;
- b) depolarizing membrane of the cell in the test sample 1;
- c) generating a current through the persistent Na<sup>+</sup> channel by adding Na<sup>+</sup> to test sample 1 at least 10 msec after step (b);
- d) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
- e) providing a control sample 1 comprising
  - i) a Na<sup>+</sup>-free physiological buffer;
  - ii) a voltage-sensitive fluorescence dye; and
  - iii) a cell having a K<sup>+</sup> channel, a transient Na<sup>+</sup> channel and a persistent Na<sup>+</sup> channel;
- f) depolarizing membrane of the cell in the control sample 1;
- g) generating a current through the persistent Na<sup>+</sup> channel by adding Na<sup>+</sup> ions to the control sample 1 at least 10 msec after step (f);
- h) detecting fluorescence emitted by the voltage-sensitive dye in the control sample 1;
- i) determining the relative emitted fluorescence 1 by comparing the emitted fluorescence from step (d) to the emitted fluorescence from step (h);
- j) providing a test sample 2 comprising
  - i) a physiological buffer;
  - ii) a voltage-sensitive fluorescence dye;
  - iii) a cell having a K<sup>+</sup> channel and a transient Na<sup>+</sup> channel; and

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers  
that Selectively Distinguish Transient from Persistent Sodium Channels

- iv) a potential Na<sup>+</sup> channel blocker
  - k) depolarizing membrane of the cell in test sample 2;
  - l) detecting the fluorescence emitted by the voltage-sensitive dye in test sample 2;
  - m) providing a control sample 2 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a K<sup>+</sup> channel and a transient Na<sup>+</sup> channel;
  - n) depolarizing membrane of the cell in control sample 2;
  - o) detecting the fluorescence emitted by the voltage-sensitive dye in control sample 2;
  - p) determining a relative emitted fluorescence 2 by comparing the emitted fluorescence from step (l) to the emitted fluorescence from step (o);
  - q) comparing the relative emitted fluorescence 1 in step (i) with the relative emitted fluorescence 2 in step (p).
12. (New): The method according to Claim 11, wherein the cell expresses an endogenous persistent Na<sup>+</sup> channel.
13. (New): The method according to Claim 11, wherein the cell expresses an exogenous persistent Na<sup>+</sup> channel.
14. (New): The method according to Claim 13, wherein the cell is HEK-293.
15. (New): The method according to Claim 11, wherein the cell expresses a Type III persistent Na<sup>+</sup> channel.
16. (New): The method according to Claim 11, wherein the membrane depolarization of step (b) is by K<sup>+</sup> addition.
17. (New): The method according to Claim 11, wherein the membrane depolarization of step (f) is by K<sup>+</sup> addition.
18. (New): The method according to Claim 11, wherein the membrane depolarization of step (b) and step (f) is by K<sup>+</sup> addition.
19. (New): The method according to Claim 11, wherein the membrane depolarization of step (b) is by field stimulation.
20. (New): The method according to Claim 11, wherein the membrane depolarization of step (f) is by field stimulation.

21. (New): The method according to Claim 11, wherein the membrane depolarization of step (b) and step (f) is by field stimulation.
22. (New): The method according to Claim 11, wherein the membrane depolarization of step (k) is by field stimulation.
23. (New): The method according to Claim 11, wherein the membrane depolarization of step (n) is by field stimulation.
24. (New): The method according to Claim 11, wherein the membrane depolarization of step (k) and step (n) is by field stimulation.
25. (New): A method for identifying a blocker of a persistent Na<sup>+</sup> channel whereby the method comprises the steps of:
  - a) providing a test sample 1 comprising
    - i) a Na<sup>+</sup>-free physiological buffer;
    - ii) a voltage-sensitive fluorescence dye;
    - iii) a cell having a K<sup>+</sup> channel, a transient Na<sup>+</sup> channel and a persistent Na<sup>+</sup> channel;  
and
    - iv) a potential Na<sup>+</sup> channel blocker;
  - b) depolarizing membrane of the cell in the test sample 1;
  - c) generating a current through the persistent Na<sup>+</sup> channel by adding Na<sup>+</sup> to test sample 1 at least 10 msec after step (b);
  - d) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
  - e) providing a control sample 1 comprising
    - i) a Na<sup>+</sup>-free physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a K<sup>+</sup> channel, a transient Na<sup>+</sup> channel and a persistent Na<sup>+</sup> channel;
  - f) depolarizing membrane of the cell in the control sample 1;
  - g) generating a current through the persistent Na<sup>+</sup> channel by adding Na<sup>+</sup> ions to the control sample 1 at least 10 msec after step (f);
  - h) detecting fluorescence emitted by the voltage-sensitive dye in the control sample 1;

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers  
that Selectively Distinguish Transient from Persistent Sodium Channels

- i) comparing the emitted fluorescence from step (d) to the emitted fluorescence from step (h).
26. (New): The method according to Claim 25, wherein the cell expresses an endogenous persistent Na<sup>+</sup> channel.
27. (New): The method according to Claim 25, wherein the cell expresses an exogenous persistent Na<sup>+</sup> channel.
28. (New): The method according to Claim 27, wherein the cell is HEK-293.
29. (New): The method according to Claim 25, wherein the cell expresses a Type III persistent Na<sup>+</sup> channel.
30. (New): The method according to Claim 25, wherein the membrane depolarization of step (b) is by K<sup>+</sup> addition.
31. (New): The method according to Claim 25, wherein the membrane depolarization of step (f) is by K<sup>+</sup> addition.
32. (New): The method according to Claim 25, wherein the membrane depolarization of step (b) and step (f) is by K<sup>+</sup> addition.
33. (New): The method according to Claim 25, wherein the membrane depolarization of step (b) is by field stimulation.
34. (New): The method according to Claim 25, wherein the membrane depolarization of step (f) is by field stimulation.
35. (New): The method according to Claim 25, wherein the membrane depolarization of step (b) and step (f) is by field stimulation.
36. (New): A method for identifying a selective blocker of a persistent Na<sup>+</sup> channel whereby the method comprises the steps of:
- a) providing a test sample 1 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a K<sup>+</sup> channel and a persistent Na<sup>+</sup> channel wherein a resting membrane potential of the cell is approximately halfway between an equilibrium potential of Na<sup>+</sup> and an equilibrium potential of K<sup>+</sup>;
  - b) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
  - c) adding a potential Na<sup>+</sup> channel blocker to test sample 1;
  - d) detecting fluorescence emitted by the voltage-sensitive dye in the test sample 1;

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers  
that Selectively Distinguish Transient from Persistent Sodium Channels

- e) determining a relative emitted fluorescence 1 by comparing the emitted fluorescence from step (b) with the emitted fluorescence from step (d);
  - f) providing a test sample 2 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye;
    - iii) a cell having a  $K^+$  channel and a transient  $Na^+$  channel; and
    - iv) a potential  $Na^+$  channel blocker
  - g) depolarizing membrane of the cell in test sample 2;
  - h) detecting the fluorescence emitted by the voltage-sensitive dye in test sample 2;
  - i) providing a control sample 2 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a  $K^+$  channel and a transient  $Na^+$  channel;
  - j) depolarizing membrane of the cell in control sample 2;
  - k) detecting the fluorescence emitted by the voltage-sensitive dye in control sample 2;
  - l) determining a relative emitted fluorescence 2 by comparing the emitted fluorescence from step (h) relative to an emitted fluorescence from step (k);
  - m) comparing the relative emitted fluorescence in step (e) with the relative emitted fluorescence in step (l).
37. (New): The method according to Claim 36, wherein the resting membrane potential of the cell is between -40 mV and -20 mV.
38. (New): The method according to Claim 36, wherein the membrane depolarization of step (g) is by field stimulation.
39. (New): The method according to Claim 36, wherein the membrane depolarization of step (j) is by field stimulation.
40. (New): The method according to Claim 36, wherein the membrane depolarization of step (g) and step (j) is by field stimulation.
41. (New): A method for identifying a blocker of a persistent  $Na^+$  channel whereby the method comprises the steps of:

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers  
that Selectively Distinguish Transient from Persistent Sodium Channels

- a) providing a test sample 1 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a  $K^+$  channel and a persistent  $Na^+$  channel wherein a resting membrane potential of the cell is approximately halfway between an equilibrium potential of  $Na^+$  and an equilibrium potential of  $K^+$ ;
  - b) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
  - c) adding a potential  $Na^+$  channel blocker to test sample 1;
  - d) detecting fluorescence emitted by the voltage-sensitive dye in the control sample 1;
  - e) comparing the emitted fluorescence from step (b) with the emitted fluorescence from step (d).
42. (New): The method according to Claim 41, wherein the resting membrane potential of the cell is between -40 mV and -20 mV.
43. (New): A method for identifying a selective blocker of a persistent  $Na^+$  channel whereby the method comprises the steps of:
- a) providing a test sample 1 comprising
    - i) a  $Cl^-$ -free physiological buffer;
    - ii) a voltage-sensitive fluorescence dye;
    - iii) a cell having a  $K^+$  channel and a persistent  $Na^+$  channel wherein a  $K^+$  conductance of the  $K^+$  channel is at least 50-fold higher than a  $Na^+$  conductance from the persistent  $Na^+$  channel; and
    - iv) a potential  $Na^+$  channel blocker;
  - b) depolarizing membrane of the cell with a Na/K pump blocker to the test sample 1;
  - c) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
  - d) providing a control sample 1 comprising
    - i) a  $Cl^-$ -free physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers that Selectively Distinguish Transient from Persistent Sodium Channels

- iii) a cell having a  $K^+$  channel and a persistent  $Na^+$  channel wherein a  $K^+$  conductance of the  $K^+$  channel is at least 50-fold higher than a  $Na^+$  conductance from the persistent  $Na^+$  channel;
  - e) depolarizing membrane of the cell with a Na/K pump blocker to the control sample 1;
  - f) detecting fluorescence emitted by the voltage-sensitive dye in the control sample 1;
  - g) comparing the emitted fluorescence from step (c) to the emitted fluorescence from step (f);
  - h) providing a test sample 2 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye;
    - iii) a cell having a  $K^+$  channel and a transient  $Na^+$  channel; and
    - iv) a potential  $Na^+$  channel blocker
  - i) depolarizing membrane of the cell in test sample 2;
  - j) detecting the fluorescence emitted by the voltage-sensitive dye in test sample 2;
  - k) providing a control sample 2 comprising
    - i) a physiological buffer;
    - ii) a voltage-sensitive fluorescence dye; and
    - iii) a cell having a  $K^+$  channel and a transient  $Na^+$  channel;
  - l) depolarizing membrane of the cell in control sample 2;
  - m) detecting the fluorescence emitted by the voltage-sensitive dye in control sample 2;
  - n) comparing the emitted fluorescence from step (j) relative to an emitted fluorescence from step (m);
  - o) comparing the difference in step (g) with the difference in step (n).
44. (New): The method according to Claim 43, wherein the Na/K pump blocker is ouabain.
45. (New): A method for identifying a blocker of a persistent  $Na^+$  channel whereby the method comprises the steps of:
- a) providing a test sample 1 comprising
    - i) a  $Cl^-$ -free physiological buffer;

Adorante, J. S., *et al.*, A High-Throughput Screen for Identifying Channel Blockers  
that Selectively Distinguish Transient from Persistent Sodium Channels

- ii) a voltage-sensitive fluorescence dye;
  - iii) a cell having a  $K^+$  channel and a persistent  $Na^+$  channel wherein a  $K^+$  conductance of the  $K^+$  channel is at least 50-fold higher than a  $Na^+$  conductance from the persistent  $Na^+$  channel; and
  - iv) a potential  $Na^+$  channel blocker;
- b) depolarizing membrane of the cell with a Na/K pump blocker to the test sample 1;
- c) detecting fluorescence emitted by the voltage-sensitive dye in test sample 1;
- d) providing a control sample 1 comprising
- i) a  $Cl^-$ -free physiological buffer;
  - ii) a voltage-sensitive fluorescence dye; and
  - iii) a cell having a  $K^+$  channel and a persistent  $Na^+$  channel wherein a  $K^+$  conductance of the  $K^+$  channel is at least 50-fold higher than a  $Na^+$  conductance from the persistent  $Na^+$  channel;
- e) depolarizing membrane of the cell with a Na/K pump blocker to the control sample 1;
- f) detecting fluorescence emitted by the voltage-sensitive dye in the control sample 1;
- g) comparing the emitted fluorescence from step (c) relative to the emitted fluorescence from step (f).
46. (New): The method according to Claim 45, wherein the Na/K pump blocker is ouabain.